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Genotype-to-Phenotype Analysis: Search for Clinical Characteristics of a Missense Change in the $GABA_A$-β1 Receptor Gene

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Genotype-to-phenotype analysis reverses the classical approach to genetic disease in which an unknown genotype is sought for a known phenotype. This paper provides an example of genotype-to-phenotype analysis for the possible psychiatric effects of a missense mutation (H396Q) at a highly conserved residue of the β1 subunit gene of the gamma aminobutyric acid type A receptor. DNA samples from 1,507 Caucasians of Western European descent were screened, and 10 heterozygotes for H396Q were identified. These individuals were matched to homozygous normal individuals by age, gender, and length of available medical records. The complete medical records of these 20 individuals were reviewed blindly by two psychiatrists (D.C.S., L.L.H.) to assess psychiatric symptomatology, with an emphasis on anxiety and related disorders. However, no association was found between this missense change at a conserved amino acid and a dominant neuropsychiatric disease phenotype. Thus, this missense change may be neutral or only mildly deleterious, may only cause recessive disease in rare individuals, or may interact epistatically with some other gene(s).

KEY WORDS: genotype-to-phenotype analysis, $GABA_A$-β1 receptor gene, anxiety disorder

INTRODUCTION

Within the new few decades, it is estimated that the full identity of the human genome will be elucidated through the Human Genome Project. In addition to revealing the human genome's 100,000 or so genes, this initiative will begin to define patterns of variation in the genome between racial and ethnic groups and, indeed, between individuals. Using conservative estimates [e.g., Lindor et al., 1994], any two individuals differ at 3×10^6 base pairs or 0.1% of their genomes. While most of this variation will not be of functional consequence, some fraction will no doubt be the substrate for individual differences in susceptibility to multifactorial disease. Every human being is believed to carry dozens of potentially deleterious mutations. However, the correlation between sequence changes and disease susceptibility and/or prognosis may not be obvious. Mechanisms may be direct (i.e., the mutation directly causes disease such as in sickle cell anemia), or indirect. Indirect mechanisms may involve the interaction of mutations in two or more genes, specific alleles altering susceptibility to environmentally mediated disease (e.g., α1-antitrypsin alleles and risk of obstructive lung disease associated with cigarette smoke), or specific alleles altering response to therapeutics (e.g., poor debrisoquine metabolism conferred by cytochrome CYP2D6 variants). The ability to infer from genotype-to-phenotype will be essential for exploiting the data gleaned on human genetic variation and for applying this information in disease prevention.

This short paper presents an initial genotype-to-phenotype analysis utilizing a recently described mutation in the gamma aminobutyric acid receptor A, β1 subunit ($GABA_A$-β1) gene [Coon et al., 1994]. Although limited in size and scope, this example illustrates the general approach and offers recommendations for more formalized approaches to genotype-to-phenotype analysis.

METHODS

The $GABA_A$ receptor is a complex composed of five subunits, including types α, β, γ, δ, and ρ [Burt and Kamatchi, 1991; Cutting et al., 1992; Barnard et al.,

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1992], but the exact subunits that comprise native $GABA_A$ receptors are not known. Of the known subunits, all but γ constitute multigene subfamilies [Burt and Kamatchi, 1991; Barnard et al., 1992]. Receptors assembled in vitro from different combinations of subunits have varied pharmacological properties (e.g., receptors with two α, two β, and one γ subunits are fully functional [D.R. Burt, personal communication]), and this variety is thought to account for the plethora of in vivo responses possible from a single neurotransmitter, GABA [Ymer et al., 1989; Sieghart, 1992; Olsen and Tobin, 1990; Barnard et al., 1992].

The $GABA_A$ receptor complex functions as a gated chloride ion channel that binds the inhibitory neurotransmitter GABA and also contains binding sites for anxiolytics (e.g., benzodiazepines), sedatives (e.g., barbiturates, alcohol), antiepileptic agents, muscle relaxants, hypnotics, and certain steroids [Burt and Kamatchi, 1991]. Alteration of GABA-ergic transmission has been hypothesized for a variety of psychiatric disorders, including anxiety disorder and schizophrenia, as well as for neurological impairments such as movement and seizure disorders. In reference to schizophrenia, a deficit in the GABA system has been suggested in schizophrenia, with findings of increased postmortem $GABA_A$ receptors [Benes et al., 1992] and decreased postmortem GABA uptake in some limbic structures of schizophrenic individuals relative to controls [Simpson et al., 1989]. Both of these findings suggest decreased GABA-ergic activity.

Recently, Coon et al. [1994] identified a putative mutation in the β1 subunit of the $GABA_A$ receptor gene. A C→G transversion in codon 396 resulted in the substitution of a glutamine residue for a histidine residue (H396Q). This amino acid is conserved in β1 subunits from rodent, bovine, and human species, as well as in the β2 rodent and bovine genes, and in the β4 avian gene. Although initially identified in a schizophrenic proband, analysis of pedigree data for linkage, and comparison of the prevalence of the mutation in multiple unique groups of unrelated schizophrenic cases and unrelated, ethnically-similar controls, did not reveal evidence for involvement of this mutation in schizophrenia. In one such comparison of schizophrenic cases and controls residing in Minnesota, two heterozygotes for the mutation were found among 140 nonschizophrenic subjects, while none were found among the 155 schizophrenics [Coon et al., 1994]. The unaffected individuals all were ascertained during the course of medical treatment through community medicine departments at the Mayo Clinic as part of a larger, case-control study of schizophrenia [Sobell et al., 1993], and all had extensive medical histories at the Mayo Clinic (average of 34.3 years).

When the medical records of the two H396Q heterozygotes were examined, it was noted that both were elderly men with histories of anxiety-related disorders. More specifically, C046 had evidence of a severe chronic anxiety disorder, uncontrolled by long-term administration of various benzodiazepines and/or antidepressants. Compilation of an extended pedigree based on Mayo Clinic and other medical records revealed that the patient's deceased mother had suffered from paranoid schizophrenia, and that her deceased identical twin sister had experienced psychotic depression. The illnesses in both women were characterized by profound somatization. The second individual (C081) found to be a carrier of the H396Q substitution did not have a formal psychiatric diagnosis, but his records and an evaluation based on the Minnesota Multiphasic Personality Inventory (MMPI) [Hathaway and McKinley, 1940] revealed an irritable, demanding personality with a strong tendency toward somatization.

Given the partially conserved nature of the substituted amino acid, the importance of GABA in anxiolytic pharmacotherapy, the hypothesized involvement of GABA in anxiety disorders, and the suggestion of psychiatric illness possibly involving anxiety in these two heterozygotes (and possibly psychosis in relatives), a genotype-to-phenotype analysis was undertaken. Ten additional heterozygotes were found from a total of 1,507 individuals screened by PCR amplification of the H396Q allele [Sommer et al., 1989, 1992], and confirmed by direct genomic sequencing [Stoflet et al., 1988].

Review and analysis of comprehensive Mayo Clinic medical records, with an emphasis on neuropsychiatric illness, were performed independently by two psychiatrists (D.C.S., L.L.H.) who were blinded to allelic status. For each identified heterozygote, a matched noncarrier of the same gender, similar age (± 5 years), Western European ethnicity, and similar initial Mayo Clinic registration date (± 1 year) (as an approximate measure of number of years of medical history) was selected. The mean age of these 10 heterozygotes was 64.0 (range 52.9–74.0) years. An average of 34.9 (range 20.1–49.1) years of medical history was available in their Mayo Clinic records. For the matched homozygous normal individuals, the average age was 63.6 (range 48.3–74.0) years; an average of 35.0 (range 20.1–49.2) years of medical history was available.

All individuals were rated for psychiatric signs, symptoms, or diagnoses on an interval scale, with "guideposts" as follows: 0, no mention of psychiatric signs/symptoms in medical history; 1, mild signs/symptoms not associated with significant work or relationship difficulties, or a very brief period (i.e., months) of more severe anxiety (e.g., panic attacks); 2, longer but still circumscribed period of significant impairment (i.e., up to a few years), as measured by work or relationship difficulties or by sense of well-being or chronic, moderate impairment in these spheres; and 3, significant lifelong impairment in work, relationships, or overall sense of well-being. The rating was referred to as an "impairment score."

RESULTS AND DISCUSSION

Twelve (60%) of the individuals had received psychotropic medications at some point in their lifetimes for various complaints, but only 5 (25%) had been evaluated by a psychiatrist. No significant difference in psychiatric impairment scores was found between carriers and matched noncarriers (Wilcoxon matched pairs signed ranks test: $P > .05$). Similarly, odds ratios for both the matched and unmatched data showed no evi-

dence of association between "affected" status (score of 2.0 or greater) and carriership of the mutation ($P > .50$). The data were reanalyzed including the two index patients (C046 and C081), with similar nonsignificant results.

The present study is an initial genotype-to-phenotype analysis of the $GABA_A$-β1 H396Q mutation. As no significant differences were found in the psychiatric ratings between heterozygotes and homozygous normal individuals, this suggests that heterozygosity for the $GABA_A$-β1 H396Q mutation is not associated with anxiety disorder or other psychiatric impairment.

However, weaknesses of this initial study include small sample size and exclusive reliance on medical histories in lieu of direct evaluation of each study subject. Had any evidence for an association between the mutation and psychiatric dysfunction emerged, all individuals would have been invited to participate in physical examinations and structured psychiatric interviews. However, given the negative results and limited resources, this extension of the study did not seem justified. With regard to the adequacy of Mayo Clinic medical histories in capturing psychiatric data, some diagnoses may well have been missed. For example, the prevalence of alcohol abuse among hospitalized patients is often underestimated if based on specific primary diagnoses of alcoholism [Baird et al., 1989]. Likewise, the results of prevalence surveys for psychiatric disorders indicate that rates are higher than those derived from data on utilization of professional services for emotional problems, indicating that a significant fraction of affected individuals never obtain professional treatment [Kessler et al., 1994]. While the shortcomings of medical records-based research is appreciated, there are several reasons to believe that the Mayo Clinic record would be more likely than other records to include psychiatric data. First, patients with diagnoses such as psychotic illnesses or major affective disorders, and especially those patients requiring medication and/or hospitalization, stand an excellent chance of being captured in the Mayo Clinic record. This is based on the facts that the Mayo Clinic maintains the only acute-care psychiatric hospital beds in the region, that regional outpatient psychiatric services are limited, and that the majority of county residents utilize the Mayo Clinic as their primary source of medical care. Second, because the Mayo Clinic operates as a large integrated practice, referrals to psychiatric services within, rather than outside of, the Mayo delivery system would be likely. These records then would be collated into the general medical record of the patient. Third, lengthy longitudinal medical records were available for the participants in the present study. These records often contained details of major life events (e.g., childbirth, death of spouse, etc.) that may be associated with certain psychiatric diagnoses (e.g., postpartum depression, grief reaction), and thus there may be an increased chance of documenting these occurrences.

In this study, the power to detect an association between specific phenotypes and carriership of the mutation was not optimal, as only two index cases and 10 additional heterozygotes were identified among the samples in our DNA bank. Statistical power will be greater in other studies if the prevalence of the mutation is higher and/or if many more individuals are available for screening.

The availability of Mayo Clinic comprehensive medical records on a large proportion of the 100,000 county residents can provide a unique resource for conducting genotype-to-phenotype studies. The establishment of a genetic epidemiology resource that includes DNA samples from a population-based sample of Mayo Clinic patients would allow the power of these types of studies to increase.

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